Journal of Basic Microbiology

Environment · Health · Techniques

22 Ping Lu et al.

Research Paper

Effects of application of corn straw on soil microbial community structure during the maize growing season

Ping Lu^{1,2}, Yin-Hua Lin¹, Zhong-Qi Yang², Yan-Peng Xu^{1,3}, Fei Tan^{1,3}, Xu-Dong Jia¹, Miao Wang⁴, De-Rong Xu⁵ and Xi-Zhuo Wang²

This study investigated the influence of corn straw application on soil microbial communities and the relationship between such communities and soil properties in black soil. The crop used in this study was maize (Zea mays L.). The five treatments consisted of applying a gradient (50, 100, 150, and 200%) of shattered corn straw residue to the soil. Soil samples were taken from May through September during the 2012 maize growing season. The microbial community structure was determined using phospholipid fatty acid (PLFA) analysis. Our results revealed that the application of corn straw influenced the soil properties and increased the soil organic carbon and total nitrogen. Applying corn straw to fields also influenced the variation in soil microbial biomass and community composition, which is consistent with the variations found in soil total nitrogen (TN) and soil respiration (SR). However, the soil carbon-to-nitrogen ratio had no effect on soil microbial communities. The abundance of PLFAs, TN, and SR was higher in C1.5 than those in other treatments, suggesting that the soil properties and soil microbial community composition were affected positively by the application of corn straw to black soil. A Principal Component Analysis indicated that soil microbial communities were different in the straw decomposition processes. Moreover, the soil microbial communities from C1.5 were significantly different from those of CK (p < 0.05). We also found a high ratio of fungal-to-bacterial PLFAs in black soil and significant variations in the ratio of monounsaturated-to-branched fatty acids with different straw treatments that correlated with SR (p < 0.05). These results indicated that the application of corn straw positively influences soil properties and soil microbial communities and that these properties affect these communities. The individual PLFA signatures were sensitive indicators that reflected the changes in the soil environment condition.

Keywords: Phospholipid fatty acid / Microbial community / Soil properties / Black soil

Received: September 14, 2013; accepted: February 7, 2014

DOI 10.1002/jobm.201300744

Introduction

Microorganisms are the most abundant and diverse organisms and are key drivers of global biogeochemical cycles [1, 2]. The soil microbial community is a key factor that influences soil resource sustainability and ecosystem function [3]. Biogeochemical cycles and the turnover processes of organic matter are determined largely by the

Correspondence: Ying-Hua Lin, Institute of Wetland Research, Chinese Academy of Forestry, Xiangshan Road, Haidian District, Beijing 100091, China

E-mail: linyinghua@263.net Phone: +86 10 62888715 Fax: +86 10 62884972

¹ Institute of Wetland Research, Chinese Academy of Forestry, Beijing, China

² Key Laboratory of Forest Protection, State Forestry Administration/Research Institute of Forest Ecology, Environment and Protection, Chinese Academy of Forestry, Beijing, China

³ College of Wildlife Resource, Northeast University of Forestry, Harbin, China

⁴ State Key Laboratory of Urban and Regional Ecology, Research Center for Eco-environmental Science, Chinese Academy of Sciences, Beijing, China

⁵ Laboratory of Bioinformatics and Noncoding RNA, Institute of Biophysics, Chinese Academy of Sciences, Beijing, China

composition and activity of soil microbial communities [4]. Therefore, soil microbial biomass, activity, and community structure are important aspects of soil quality [5]. Due to the sensitivity of soil microorganisms to changes in soil organic matter, the change in soil microbial community composition can be among the earliest indicators of soil health in many ecosystem processes [6]. Previous studies have shown that soil microbial community structure was influenced easily by environmental factors, such as quantity and quality of available soil carbon [7], nitrogen [8], texture [9], land-use change [10, 11], and season [12, 13]. Crop and soil management practices, such as the application of organic manure and inorganic fertilizers, may influence soil microbial biomass and activity [14].

Fertilization, as a common and important agricultural management practice, is used primarily to increase soil nutrients and provide essential nutrients for crop growth, but it can also affect soil microbial community structure, improve the molecular diversity of soil microbial [15-17], and improve soil fertility by influencing the diversity and activity of soil microbial [18]. Straw, as an important organic fertilizer and renewable resource, contains abundant C, N, P, K, and other nutrients and is readily available [19–21]. Approximately 3.4 billion tons of crop straw residues are produced every year in the world, 0.47 billion tons of which are maize; 97% were burned, stacked, and abandoned, which is a waste of resources and contributes to environmental pollution [20, 22]. China is one of most abundant straw resources in the world, producing more than 0.6 billion tons of straw every year, which represents approximately 33-45% of energy consumption for livelihood in rural areas [23]. The rational use of straw resources is becoming an inevitable requirement for sustainable development of agriculture in China [24]. Returning straw residue to the fields is a primary way to use straw effectively and is an important measure to improve the basic fertility of farmland soil [25]. Returning straw to the soil has been used to increase soil organic matter, improve soil physical properties, and increase crop productivity [26, 27]. Additionally, the application of straw has been used to improve the activity of soil microbial and promote soil nitrogen and carbon sequestration potential [28-30].

This study selected black soil to study the influence of straw application on soil microbial compositions. Black soil is one of the most important soil resources in Northeast China and is one of the most fertile and best texture soils. The objectives of this study were (1) to analyze the composition of the soil microbial community in black soil after the application of corn straw, and (2) to analyze the relationships between the microbial commu-

nity and specific soil properties, i.e., soil moisture, temperature, pH, organic carbon, and total nitrogen.

Materials and methods

Soil sampling and experimental design

This experiment was conducted in the greenhouse of the Chinese Academy of Forestry, Beijing. Throughout the experiment, the temperature was maintained at 25 °C. The soil selected for this study was obtained from the southern black soil area (40°52′-46°18′N, 121°38′-131° 19′E) in the Gongzhuling experiment base of Jilin Academy of Agricultural Sciences. The soil pH was 7.2, soil organic carbon and total nitrogen contents were 17.46 and 1.47 g kg $^{-1}$, respectively, and soil available nitrogen content was 116.76 mg kg $^{-1}$. The soils were root-picked and sieved with a 4 mm mesh sieve before use.

The corn straw was composed primarily of cellulose containing 42% organic carbon. Corn straw applications consisted of a gradient of four levels of application: 50, 100, 150, and 200% of shattered corn straw residue (referred to as C0.5, C1.0, C1.5, and C2.0, respectively). According to the local conventional fertilization with corn straw (7500 kg ha^{-1}), the treatments were applied as follows: C0.5 plots received carbon as corn straw at $1.54\,\mathrm{g\,kg^{-1}}$ soil; C1.0 plots received carbon as corn straw at 3.08 g kg⁻¹ soil; C1.5 plots received carbon as corn straw at $4.62\,\mathrm{g\,kg^{-1}}$ soil; C2.0 plots received carbon as corn straw at 6.16 g kg⁻¹ soil, and the control plot (CK) remained unfertilized. Each clay pot (30 cm in diameter and 40 cm in height) was filled with 25 kg soil. Corn straw was applied one-time to each pot, and maize (Zea mays L.) was planted in each pot on April 20, 2012. Each pot containing one corn plant was assigned randomly to one of the treatments, and each treatment was repeated three times (three pots per treatment).

Sampling was conducted on May 20, June 20, July 20, August 20, and September 20, 2012, roughly at the seedling, jointing, silking, grain-filling, and maturity stages of the plants, respectively. Soil samples were collected from 0–20 cm depths in each plot with a pipe 1 cm in diameter. The samples used for phospholipid fatty acid (PLFA) measurement were stored at $-80\,^{\circ}\text{C}$ and the remaining soil was used for soil property measurements after air drying.

Soil analysis

Soil moisture (SM) and temperature (ST) were measured directly by soil hygrometer BSG-T300 in the plotted soil. Soil pH was determined through a suspension sample with a soil (air-dried) to water (w:v) ratio of 1:2.5 and

24 Ping Lu et al.

measured with a digital pH meter. Soil organic carbon (SOC) was determined by dichromate oxidation, and total nitrogen (TN) was measured using the semi-micro Kjeldahl method [31]. Soil respiration (SR) was determined by the MicroRespTM approach [32].

Phospholipid fatty acids (PLFA) analysis

The structure of the soil microbial community was assessed by phospholipid fatty acids (PLFA) analysis using the method described by Bligh and Dyer [33] and Frostegård *et al.* [34]. The lipid were extracted and then identified with a standard qualitative mix ranging from C10 to C20 and the MIDI peak identification system (Microbial ID. Inc., Newark, DE). For each sample, the abundance of individual fatty acid methyl-esters was expressed in nmol PLFA g^{-1} dry soil.

The fatty acid nomenclature used was that described by Frostegård et al. [34]. Concentrations of each PLFA were calculated based on the 19:0 internal standard concentrations. The relative abundance of an individual fatty acid was expressed as the proportion (mol%) of the sum of all fatty acid [14, 35]. Gram-positive bacteria were identified by the PLFAs: i14:0, i15:0, i16:0, i17:0, a15:0, a17:0, Gram-negative bacteria were identified by the PLFAs: $16:1\omega7c$, $17:1\omega8c$, cy17:0, $18:1\omega5c$, cy19:0, and non-specific bacteria were identified by the saturated straight-chain PLFAs: 14:0, 15:0, 16:0, 18:0 [36]. The fungi were identified by the PLFA $18:2\omega6c$ [34, 37, 38], and PLFAs 16:1ω5c were used as a marker for arbuscular mycorrhizal fungi (AMF) [39, 40]. A ratio of the sum of monounsaturated fatty acids to the sum of branched fatty acids (M/B) (16:1ω5c, 16:1ω7c, 17:1ω8c, 18:1ω5c, 18:1ω9c/ i15:0, i16:0, i17:0, a15:0, a17:0) was used to indicate the relative ratio of aerobic to anaerobic organisms [41], while the ratio of fungal-to-bacterial PLFAs ($18:2\omega6c/i14:0$, i15:0, a15:0, 15:0, i16:0, i17:0, a17:0, $16:1\omega7c$, cy17:0, cy19:0) was used as an indicator of changes in the relative abundance of these two microbial groups [34, 37, 42].

Data analysis

One-way and multivariate ANOVAs for differences in soil properties and the soil microbial community with

different sampling times and straw application treatments were performed with SPSS 19.0 (SPSS Institute, Inc., 2010), and a LSD test was used to carry out multiple *post hoc* comparisons. Before analysis, the data were natural-log transformed where necessary to improve normality and homogeneity of variance. Significant differences were set as p < 0.05. Additionally, correlations between soil properties and microbial variables were determined using Pearson correlation coefficients.

Microbial biomass was calculated as the sum of the individual PLFAs (nmol g⁻¹ soil). PLFAs that contributed less than 1% of the total amount extracted from each sample, or those that were observed in only one sample were eliminated from the data set, yielding 19 PLFAs for statistical analysis. The composition of the soil microbial community was summarized using a principle component analysis (PCA) on the relative abundance (mol%) of PLFAs in each sample. PCA was conducted using CANOCO software (Canoco for Windows 4.5). Redundancy analysis (RDA) as a direct ordination technique based on PCA was used to test specific hypotheses about the relationships between soil properties and microbial community composition. Soil properties were tested for significant contribution to the explanation of the variation in the PLFA data with the Monte Carlo permutation test based on 999 random permutations (p < 0.05) [43]. Soil properties that were significantly correlated with factors in the RDA were stressed in the plots. Soil properties were represented by vectors. Vectors of greater magnitude and forming smaller angles with an axis are more strongly correlated with that axis [42, 44].

Results

Soil properties

The soil pH, SM, ST, SR, TN, SOC, and C/N are given in Table 1. By comparison to the control treatment (CK), ST, soil pH, TN, SOC, C/N, and SR increased with straw application (Table 1). ST and SM increased from May to August, while TN, SOC, pH, and SR decreased from May to

Table 1. Soil properties under different straw treatments.

Treatments	ST (°C)	SM (%)	pH (1:2.5H ₂ O)	TN (g kg ⁻¹)	SOC (g kg ⁻¹)	C/N ^a	SR (μg CO ₂ –C g ⁻¹ h ⁻¹)
CK	26.53 ± 2.82	53.91 ± 12.77	$\textbf{7.04} \pm \textbf{0.20}$	1.45 ± 0.05	15.77 ± 1.27	10.88 ± 0.97	83.96 ± 12.65
C0.5	26.58 ± 2.70	56.04 ± 10.87	7.03 ± 0.22	$\boldsymbol{1.47 \pm 0.05}$	15.75 ± 0.95	10.73 ± 0.52	84.72 ± 14.27
C1.0	26.82 ± 2.69	53.98 ± 12.42	7.02 ± 0.23	1.46 ± 0.04	16.02 ± 0.84	10.94 ± 0.49	90.04 ± 24.85
C1.5	27.07 ± 2.58	52.81 ± 12.94	$\boldsymbol{7.08 \pm 0.26}$	$\boldsymbol{1.48 \pm 0.07}$	16.18 ± 0.76	10.94 ± 0.34	93.46 ± 32.20
C2.0	26.69 ± 2.58	55.96 ± 11.35	$\boldsymbol{7.06 \pm 0.21}$	$\boldsymbol{1.50 \pm 0.05}$	$\textbf{16.39} \pm \textbf{1.12}$	$\boldsymbol{10.92 \pm 0.77}$	85.99 ± 11.25

^aC/N refer to the ratio of soil organic carbon to soil total nitrogen, respectively.

Table 2. F-test for months and treatments of soil microbial composition and soil properties (P values).

	Months	Treatments	$Months \times treatments$
Total PLFA	NS ^a	0.021	0.008
b	0.027	NS	NS
AMF	0.007	0.000	NS
G+	0.001	NS	0.041
M/B	0.000	NS	NS
ST	0.000	NS	NS
SM	0.000	NS	NS
SR	0.000	0.000	0.000
pН	0.000	NS	NS
SOC	0.000	NS	NS
TN	0.000	0.019	0.038
C/N	0.001	NS	NS

b, bacterial PLFAs; AMF, arbuscular mycorrhizal fungi PLFAs; G+, Gram-positive bacterial PLFAs; M/B, the ratio of the sum of monounsaturated fatty acids to the sum of branched fatty acids. $^{\rm a}$ NS, not significant at p < 0.05.

August and increased in September. Multifactor ANOVA showed that SR (p < 0.01) and TN (p < 0.05) were significantly affected by treatments and months. The month effect on ST, SM, SR, pH, TN, SOC, and C/N was significant (p < 0.01), and the treatment effect on SR (p < 0.01) and TN (p < 0.05) was significant (Table 2). However, TN and SR were both greater in C1.5 than in other treatments (Table 1).

Soil microbial structural diversity

Total PLFA was affected significantly by the levels of straw application and month (p < 0.01; Table 2). By comparison to the control treatment (CK), total PLFA was higher with the straw application treatments. The straw application treatments in total PLFA was ranked in the order: C1.5 (46.21 nmol g⁻¹) > C2.0 (44.01 nmol g⁻¹) > C1.0 (42.11 nmol g⁻¹) > C0.5 (41.02 nmol g⁻¹). The total PLFA was the highest in C1.5 from July to September.

The relative abundance of the saturated fatty acids, bacterial PLFAs, Gram-negative bacterial PLFAs, Grampositive bacterial PLFAs, fungal PLFAs, AMF PLFAs, the ratio of fungal-to-bacterial PLFAs (F/B), and the ratio of the sum of monounsaturated fatty acids to the sum of branched fatty acids (M/B) are shown in Fig. 1. The proportional abundance of Gram-positive bacterial PLFAs was affected significantly by treatments and months (p < 0.05); AMF PLFAs were significantly different by treatments (p < 0.01) and month (p < 0.01), and bacterial PLFAs and M/B were significantly different over months (p < 0.05), but not across treatments (Table 2). In comparison to CK, the relative abundances of the saturated fatty acids, AMF PFLAs, fungal PLFAs, bacterial PLFAs, Gram-negative bacterial PLFAs and Gram-positive bacterial PLFAs were higher under the straw application treatments.

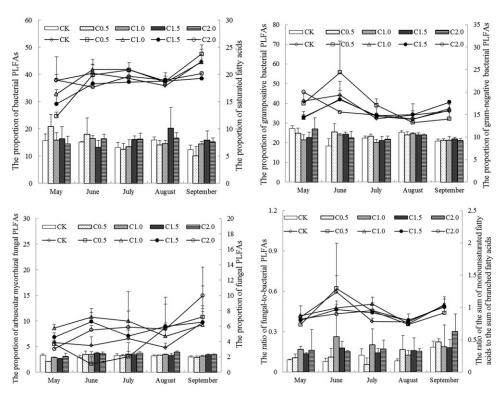


Figure 1. The relative abundances of individual PLFAs (mol%) with different straw application treatments at different times. The columns correspond to the left *y* coordinate and the line corresponds to the right *y* coordinate.

26 Ping Lu et al.

The mean abundances of fungal PLFAs and saturated fatty acids were higher in C1.0 ($6.09\pm0.95\%$ and $19.81\pm2.32\%$) compared to that in other treatments, and they increased from May to September, whereas the mean abundances of bacterial PLFAs and Gram-positive bacterial PLFAs were lower in C1.0 ($15.09\pm1.17\%$ and $22.17\pm1.92\%$) compared to that in other treatments, and decreased from May to September. However, the proportions of bacterial PLFAs were significantly higher than those of fungal PLFAs in all treatments (p < 0.01; Fig. 1).

The relationship between soil microbial community composition and soil properties

The total PLFA was correlated positively with SR and TN (p < 0.05). The bacterial PLFAs were correlated negatively with SM (p < 0.05), the Gram-positive bacterial PLFAs and M/B were both correlated with SR (p < 0.05). The Gramnegative bacterial PLFAs were correlated positively with pH and SOC (p < 0.05), as well as with TN (p < 0.01; Table 3).

In the Principal Components Analysis (PCA) of the microbial community composition defined by the PLFA profile, the first two axes explained 53.3 and 15.9% of the total variation in microbial communities, respectively. Consistent with the pattern seen in the PCA plot, the microbial community was correlated significantly with the first (r = 0.67, p < 0.01) and second (r = 0.42, p < 0.01)axis. Consistent with the patterns seen in the PCA plot, the first axis corresponded to both the months and the treatments, even when values on the first axis corresponded to the months (p < 0.001) more strongly than to treatments (p > 0.05). The microbial communities in C1.5 and CK were separated from each other on the first PCA axis (p < 0.05), and these two sites were set apart from the month on the origin of the PCA axis (p < 0.05). Both months (p < 0.01) and treatments (p < 0.01) corresponded strongly to the second axis. On the second axis, the

Table 3. Pearson correlation coefficients between soil microbial composition and soil properties.

	Total PLFA	b	G+	G-	M/B
ST	-0.032	⁻ 0.146	-0.144	-0.223	-0.009
SM	-0.105	-0.272^{*}	-0.192	-0.209	-0.022
pН	-0.013	0.119	0.055	0.261^{*}	0.146
TN	0.247^{*}	0.223	0.203	0.297^{**}	0.133
SOC	0.164	0.195	0.152	0.295^{*}	0.133
C/N	0.011	0.062	0.029	0.122	0.057
SR	0.233^{*}	0.179	0.293^{*}	0.129	-0.246^{*}

p < 0.05. **p < 0.01.

microbial communities in C1.5 and C0.5 were separated distinctly from each other (p < 0.01), with C1.5 below the origin of the PCA axis, while C0.5 was above the origin (Fig. 2).

The relationship between the microbial community composition and soil properties was analyzed by RDA. The first two axes explained 73.9 and 16.9% of the total variation in microbial communities, respectively. The significance of environmental variables (ST, SM, soil pH, SOC, TN, C/N, and SR) presented in the ordination was determined by Monte Carlo permutation tests (p < 0.05). The results showed that seven environmental variables were significant on the first axis (F = 21.12, p < 0.01) and all canonical axes (F = 4.60, p < 0.01). The variations in PLFA profiles were influenced predominantly by ST (F = 11.03, p < 0.01), SR (F = 8.35, p < 0.01), soil pH (F = 5.07, p < 0.01), and SM (F = 2.84, p < 0.05). Changes in microbial community composition along axis 1 were associated with both higher values of pH and lower values of ST and SM (Fig. 3). The microbial communities collected from May and June were associated positively with relatively higher soil pH, while samples collected from July, August, and September were associated positively with ST and SM (Fig. 3).

Discussion

The effect of corn straw application on soil

Application of corn straw can reduce fertilizer use and production costs, while also reducing the environmental pollution caused by burning straw, which is a method used frequently to remove residues in fields [20, 45]. Applying straw to the soil can also improve soil physical properties and promote effective decomposition of humus by increasing the diversity and activity of soil microbial, thus contributing to a virtuous cycle of agricultural production [20, 26, 27]. In this study, by comparison to CK, soil properties were influenced significantly and positively by the application of corn straw [18, 46]. Corn straw applied to the soil tillage layer (0-20 cm) had a higher thermal radiation than the soil [47], which can improve soil bulk density and total porosity that enhances soil water holding capacity and infiltration [48]. In corn straw application treatments, the soil temperature, moisture, and respiration were 0.19-2.03, 0.13-3.95, and 0.91-11.31% higher than those in CK, respectively. Currently, inputs of soil nutrients depend primarily on the use of expensive and potentially toxic chemical fertilizers, while a large amount of crop straw is discarded or burned, thus resulting in declines in soil organic carbon content in farmland ecosystems on

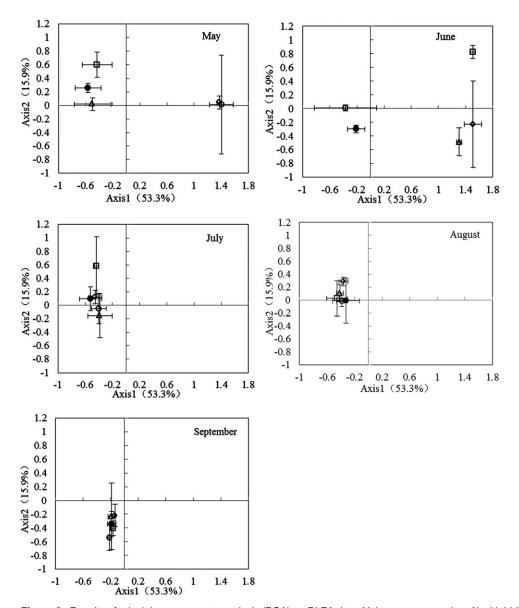


Figure 2. Results of principle components analysis (PCA) on PLFA data. Values are means (n=3) with bidirectional error bars of axis 1 and 2. For all PCA plots, values on the x and y axes represent the percent variation explained by axis 1 and 2, respectively. \Diamond CK; \Box C0.5; \triangle C1.0; \bigcirc C2.0.

the time-scales [49]. In previous studies, direct returning of straw to soil increased soil organic carbon stocks and soil nitrogen content in agricultural soils, thereby improving soil quality [27, 50]. In this study, soil organic carbon and total nitrogen content in the corn straw application treatments were higher than those in CK, and both were highest in C2.0. These results suggest that applying corn straw has obvious effects not only on holding soil water, regulating soil temperature, and improving soil bulk density but also on increasing soil carbon storage and transformation in agricultural soils [50].

Soil microbial is sensitive to the environment variation, resulting in soil microbial community would also be influenced [41, 51]. In this study, by compare to CK, total PLFA, the relative abundances of the saturated fatty acids, AMF PLFAs, fungal PLFAs, bacterial PLFAs, Gram-negative bacterial PLFAs, and Gram-positive bacterial PLFAs were increased under the corn straw application (Fig. 1). Total PLFA and AMF PLFAs in C1.5 and C2.0 were significantly different with that in CK (p < 0.05); Gram-positive bacterial PLFAs in C1.5 was significantly different with that in CK (p < 0.05); fungal PLFAs in C2.0 was significantly different with that in C0.5 (p < 0.05). Our

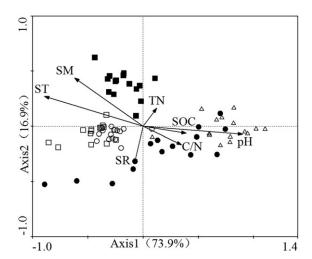


Figure 3. Redundancy analysis (RDA) results of microbial community composition and soil properties. Soil properties that were correlated significantly with factors in the RDA were stressed in the plots (Monte Carlo permutation tests, p < 0.05). Vectors represented the mean value of the soil properties and the mean abundances of microbial community. The direction of an arrow indicates the steepest increase in the variable and the length indicates the strength relative to the other variables. For all RDA plots, values on the x and y axes represented the percent variation explained by axis 1 and 2, respectively (p < 0.05). lacktriangle May; \triangle June; \bigcirc July; \square August.

results validated that the application of corn straw was essential for improving soil quality and increasing the diversity of soil microbial.

Soil microbial community and soil properties

Soil properties also played an important role in explaining variation in soil microbial community composition [51]. Total PLFA was influenced by the interaction of the sampling times and straw application treatments, which were correlated with the variations in soil TN and SR (Table 3 and Figs. 2 and 3). Higher total PLFA in C1.5 was related to higher TN and SR, and lower total PLFA in C0.5 was related to lower TN and SR (Tables 1 and 3), indicating that soil properties might account for the variation in total PLFA.

High soil fertility favored soil microbial growth [52, 53]. Different soil types were associated with different soil properties, which might contribute to the conflicting correlations to total PLFA with SM, ST, and SOC [42, 54, 55]. Soil N, which can be increased by the application of corn straw residues, is the most important source of nutrients for microbial utilization [27]. In this study, total PLFA was correlated positively with TN (Table 3), and TN was influenced by different straw application treatments (Table 1), which suggested that soil microorganisms were influenced by corn straw application [56]. However, we did not find significant correlations between total PLFA

and ST or SM, while ST and SM were not significantly different under the straw application treatments (Tables 2 and 3) because the experiment was conducted in a greenhouse with constant temperature and humidity. Although we did not find a significant correlation between total PLFA and SOC, we did find a positive correlation between Gram-negative bacterial PLFAs and SOC (Table 3), and therefore a more pertinent reason for such a phenomenon merits further investigation.

Soil respiration (SR) is a fundamental biochemical process for the decomposition of soil organic carbon, and can reflect the extent of the decomposition of soil organic matter and the level of nutrition supplied; thus, it can be used as an index for the evaluation of the total activity of the microorganisms [57]. The application of straw residues can improve soil aeration conditions, which will enhance soil microbial growth and activity [27, 58]. Soil microbial activity is also the main source of soil respiration. In the present study, soil respiration increased significantly with the application of increased amounts of straw (Table 1) [59, 60], and influenced the variation in PLFA profiles (Table 3 and Fig. 3), which suggested that soil microorganisms were influenced by corn straw application [56].

The soil microbial community increases generally with pH and soil moisture, and the increased soil pH has been shown to cause the soil microbial community to change from fungi-dominated to bacteria-dominated [61, 62]. In previous studies, soil microbial community composition was correlated highly with soil pH [37, 63]. Those studies found that bacterial PLFA increased with increasing soil pH, and it was suggested that pH controlled the soil microbial community composition [63-65]. In our study, Gram-negative bacterial abundance was correlated positively with pH (Tables 1 and 3; Fig. 1). The soil microbial community composition was correlated positively with soil pH from May to June, but correlated negatively from July to September, which suggests that soil pH as the constraint on soil microorganisms was restricted by other environmental variables [66].

Soil C/N has been observed to be the best predictor of the soil microbial community, with fungal biomass being high when there is a high C/N, and bacterial biomass increasing when this ratio declines [61]. In our study, fungal PLFA was correlated negatively (r = -0.54, p < 0.05) with C/N in May, bacterial PLFA, Gram-positive bacterial and Gram-negative bacterial PLFAs were correlated positively (r = 0.63, 0.64, 0.59, 0.62, p < 0.05) with C/N in September. However, we did not find significant correlations between fungal, bacterial PLFA and C/N in June, July, and August, and we also did not find

a significant correlation between total PLFA and C/N (Table 3 and Fig. 3) [42].

Indicator of soil microbial community to soil environment condition

It is very important to provide organic matter for soil to soil organic carbon content, soil texture, soil biodiversity, and food chain [67], and corn straw application could contain soil organic carbon and improve soil fertility and the activity of soil microbial [46, 68]. In this study, SOC and TN increased with increasing levels of corn straw application (Table 1). The nutrients released by different treatments had different effects on the soil microbial community (Fig. 1 and Table 3). It may be that the seedling stage (May) of the corn plant needed less nutrition than the maturity stage (September); there were also more nutrients for the soil microbial community in May than in September. Therefore, higher total PLFA was found in May (45.29 nmol g⁻¹ \pm 9.92), and lower total PLFA on September $(39.75 \, \text{nmol g}^{-1} \pm 6.28).$

It has been found that individual signature PLFAs may be sensitive indicators of improvement in soil abiotic conditions [69, 70]. Arbuscular mycorrhizal fungi (AMF) play an important role in nutrient cycling and in improving structural soil characteristics [71–73]. AMF PLFAs was more abundant in C2.0 in August, and fungal PLFAs were more abundant in C2.0 in September, which may be associated with high SOC in C2.0 because fungi incorporates more soil carbon into biomass than does bacteria and the carbon turnover is slower in fungidominated ecosystems [42].

The ratio of fungal-to-bacterial PLFAs is used commonly as an indicator of changes in the relative abundance of these two microbial groups in soil microbial communities [34, 37]. The ratios of fungal-to-bacterial PLFAs were higher in this study compared with the findings in other studies [37, 51], indicating that fungi predominated in our treatments. This conclusion is consistent with previous reports of a high fungal-to-bacterial ratio in agricultural soils [74]. The predominance of fungi over bacteria in our soils might be due to the higher SOC in agricultural soils [34, 74].

M/B is used as an indicator of the relative ratio of aerobic to anaerobic organisms to slow-growing anaerobic bacteria. The higher M/B with fast-growing aerobic bacteria takes place under condition of better soil aeration, while the lower M/B with slow-growing anaerobic bacteria occurs in more poorly aerated soil [41]. In our study, M/B was correlated significantly with SR (p < 0.05) which was used commonly as an indicator of soil quality. In addition, M/B decreased with increased

straw applications (except for C0.5), suggesting that soil aeration was poorer in C2.0 compared with that in C1.0 and C1.5. However, the M/B increased from May to September (except for August) in C2.0, suggesting that M/B shifted over months in C2.0. It suggested that these individual signature PLFAs were sensitively responded to application of corn straw, and might be regarded as sensitive indicator of soil environment condition.

In summary, application of corn straw can influence soil properties to improve soil quality. The variation in soil microbial community compositions with straw application treatments corresponded to the variance in sampling times, which correlated with the amounts of soil TN and SR. The number and abundances of PLFAs with the amounts of soil TN and SR were higher in C1.5 compared with those in C0.5, C1.0, and C2.0, which indicated that the structural diversity of the soil microbial community was affected positively by the application of corn straw. However, the changes of PLFA profiles in black soil with different straw application treatments provided some evidence of changes in soil conditions. Therefore, further studies should address the long-term effects of crop yield on ecologically relevant processes.

Acknowledgments

This work was supported by the National Natural Science Foundation of China (31071904). We thank Professor Chunyu Shi and Professor Junsheng Li for help and advice in designing the experiments.

References

- [1] Torsvik, V, Øvreås, L, Thingstad. T.F, 2002. Prokaryotic diversity-magnitude, dynamics, and controlling factors. Science, 296, 1064–1066.
- [2] Falkowski, P.G, Fenchel, T, Delong. E.F, 2008. The microbial engines that drive Earth's biogeochemical cycles. Science, 320, 1034–1039.
- [3] Sparling, G.P. 1997. Soil microbial biomass, activity and nutrient cycling as indicators of soil health, in: Pankhurst, C.E., Doube, B.M., Gupta, V.V.S.R. (Eds.), Biological Indicators of Soil Health, CAB International, UK, 97–119.
- [4] Zelles, L., 1999. Fatty acid patterns of phospholipids and lipopolysaccharides in the characterization of microbial communities in soil: a review. Biol. Fertil. Soils, **29**, 111–129.
- [5] Schloter, M., Dilly, O., Munch, J.K., 2003. Indicators for evaluating soil quality. Agric. Ecosyst. Environ., 98, 255–262.

- [6] Dilly, O., Munch, J.C., 1998. Ratios between estimates of microbial biomass content and microbial activity in soils. Biol. Fertil. Soils, 27, 374–379.
- [7] Blaalid, R., Carlesen, T., Kumar, S., Halvorsen, R. et al., 2012. Changes in the root-associated fungal communities along a primary succession gradient analysed by 454 pyrosequencing. Mol. Ecol., 21, 1897–1908.
- [8] Eaton, W.D., Mcdonald, S., Roed, M., Vandecar, K.L. et al., 2011. A comparison of nutrient dynamics and microbial community characteristics across seasons and soil types in two different old growth forests in Costa Rica. Trop. Ecol., 52, 35–48.
- [9] Monroy, F., van der Putten, W.H., Yergeau, E., Mortimer, S.R. et al., 2012. Community patterns of soil bacteria and nematodes in relation to geographic distance. Soil Biol. Biochem., 45, 1–7.
- [10] Wang, X.L., Jia, Y., Li, X.G., Long, R.J. et al., 2009. Effects of land use on soil total and light fraction organic, and microbial biomass C and N in a semi-arid ecosystem of northwest China. Geoderma, 153, 285–290.
- [11] Drenovsky, R.E., Steenwerth, K.L., Jackson, L.E., Scow, K.M., 2010. Land use and climatic factors structure regional patterns in soil microbial communities. Global Ecol. Biogeogr., 19, 27–39.
- [12] Smit, E., Leeflang, P., Gommans, S., van Den Broek, J. et al., 2001. Diversity and seasonal fluctuations of the dominant members of the bacterial soil community in a wheat field as determined by cultivation and molecular methods. Appl. Environ. Microbiol., 67, 2284–2291.
- [13] Williams, M.A., 2007. Response of microbial communities to water stress in irrigated and drought-prone tallgrass prairie soils. Soil Biol. Biochem., 39, 2750–2757.
- [14] Böhme, L., Langer, U., Böhme, F., 2005. Microbial biomass, enzyme activities and microbial community structure in two European long-term field experiments. Agric. Ecosyst. Environ., 109, 141–152.
- [15] Chander, K., Goyal, S., Mundra, M.C., Kapoor, K.K., 1997. Organic matter, microbial biomass and enzyme activity of soils under different crop rotations in the tropics. Biol. Fertil. Soils, 24, 306–310.
- [16] Zhang, P.J., Li, L.Q., Pan, G.X., Zhang. J.W., 2004. Influence of long-term fertilizer management on topsoil microbial biomass and genetic diversity of a paddy soil from the Tai Lake region, China (in Chinese). Acta Ecol. Sin., 24, 2818– 2824.
- [17] Liu, X.Z., Zhang, L.M., Prosser, J.I., He, J.Z., 2009. Abundance and community structure of sulfate reducing prokaryotes in a paddy soil of southern China under different fertilization regimes. Soil Biol. Biochem., 41, 687– 694.
- [18] Sun, R.L., Zhu, L.S., Zhao, B.Q., Zhou, Q.X. et al., 2004. Effects of long-term fertilization on soil microorganism and its role in adjusting and controlling soil fertility (in Chinese). Chin. J. Appl. Ecol., 15, 1907–1910.
- [19] Lao, X.R., Sun, H.W., Wang, Z., Hao, Y.R. et al., 2003. Effect of matching using of straw and chemical fertilizer on soil fertility (in Chinese). Acta Pedol. Sin., 40, 618–623.
- [20] Shen, Y.Y., Chen, H., 2009. The progress of study on soil improvement research with straw stalk (in Chinese). Chin. Agric. Sci. Bull., 25, 291–294.

- [21] Xu, R.L., Wang, J.F., Zhang, G.L., Dai, Q.G., 2010. Changes of microbe and organic matter content in paddy soil applied with straw, manure and nitrogen fertilizer (in Chinese). Acta Ecol. Sin., 30, 3584–3590.
- [22] Lal, R., 1997. Residue management, conservation tillage and soil restoration for mitigating greenhouse effect by CO₂-enrichment. Soil Tillage Res., 43, 81–107.
- [23] Zeng, X.Y., Ma, Y.T., Ma. L.R., 2007. Utilization of straw in biomass energy in China. Renew. Sust. Energy Rev., 11, 976–987.
- [24] Wang, Y.J., Bi, Y.Y., Gao, C.Y., 2010. The assessment and utilization of straw resources in China. Agric. Sci. Chin., 9, 1807–1815.
- [25] Bi, Y.Y., Wang, D.L., Gao, C.Y., Wang, Y.J. (Eds.), 2008. Evaluation and Utilization of Crop Straw Resources in China, China Agricultural Science and Technology Press, Beijing, 78–79.
- [26] Liu, E.K., Yan, C.R., Mei, X.R., He. W.Q. et al., 2010. Long-term effect of chemical fertilizer, straw, and manure on soil chemical and biological properties in northwest China. Geoderma, 158, 173–180.
- [27] Lou, Y.L., Liang, W.J., Xu, M.G., He. X.H. et al., 2011. Straw coverage alleviates seasonal variability of the topsoil microbial biomass and activity. Catena, 86, 117–120.
- [28] Mary, B., Recous, S., Darwis, D., Robin. D., 1996. Interactions between decomposition of plant residues and nitrogen cycling in soil. Plant Soil, 181, 71–82.
- [29] Recous, S., Aita, C., Mary, B., 1999. In situ changes in gross N transformations in bare soil after addition of straw. Soil Biol. Biochem., 31, 119–133.
- [30] Lu, F., Wang, X.K., Han, B., Ouyang, Z.Y. et al., 2009. Soil carbon sequestrations by nitrogen fertilizer application, straw return and no-tillage in China's cropland. Global Change Biol., 15, 281–305.
- [31] Dong, M. (Ed.), 1996. Survey, Observation and Analysis of Terrestrial Biocommunities, China Standard Press, Beijing.
- [32] Campbell, C.D., Chapman, S.J., Cameron, C.M., Davidson, M.S. et al., 2003. A rapid microtiter plate method to measure carbon dioxide evolved from carbon substrate amendments so as to determine the physiological profiles of soil microbial communities by using whole soil. Appl. Environ. Microbiol., 69, 3593–3599.
- [33] Bligh, E.G., Dyer. W.J., 1959. A rapid method of total lipid extraction and purification. Can. J. Biochem. Physiol., 37, 911–917.
- [34] Frostegård, A., Bååth, E., 1996. The use of phospholipid analysis to estimate bacterial and fungal biomass in soils. Biol. Fertil. Soils, 22, 59–65.
- [35] Zelles, L., Bai, Q.Y., Beck, T., Beese. F., 1992. Signature fatty acids in phospholipids and lipopolysaccharides as indicators of microbial biomass and community structure in agricultural soils. Soil Biol. Biochem., 24, 317–323.
- [36] Zelles, L., 1997. Phospholipid fatty acid profiles in selected members of soil microbial communities. Chemosphere, 35, 275–294.
- [37] Bååth, E., Anderson, T.H., Comparison of soil fungal/bacterial ratios in a pH gradient using physiological and PLFA-based techniques. Soil Biol. Biochem., 35, 955–963.

- [38] Klamer, M., Bååth, E., 2004. Estimation of conversion factors for fungal biomass determination in compost using ergosterol and PLFA 18:2ω6,9. Soil Biol. Biochem., 36, 57–65.
- [39] Olsson, P.A., Bååth, E., Jacobsen, I., Soderstrom, B., 1995. The use of phospholipid and neutral lipid fatty acids to estimate biomass of arbuscular mycorrhizal fungi. Mycol. Res., 99, 623–629.
- [40] Madan, R., Pankhurst, C., Hawke, B., Smith, S., 2002. Use of fatty acids for identification of AM fungi and estimation of the biomass of AM spores in soil. Soil Biol. Biochem., 34, 125–128.
- [41] Bossio, D.A., Fleck, J.A., Scow, K.M., Fujii, R., 2006. Alteration of soil microbial communities and water quality in restored wetlands. Soil Biol. Biochem., 38, 1223–1233.
- [42] Cao, Y.S., Fu, S.L., Zou, X.M., Cao, H.L. et al., 2010. Soil microbial community composition under Eucalyptus plantations of different age in subtropical China. Eur. J. Soil Biol., 46, 128–135.
- [43] Manly, B.F.J. (Ed.). 2006. Randomization, Bootstrap and Monte Carlo Methods in Biology, Chapman & Hall/CRC, London.
- [44] Zornoza, R., Guerrero, C., Mataix-Solera, J., Scow, K.M. et al., 2009. Changes in soil microbial community structure following the abandonment of agricultural terraces in mountainous areas of Eastern Spain. Appl. Soil Ecol., 42, 315–323.
- [45] Zhang, T.T., Huang, J., Deng, S.B., Yu, G. 2011. Influence of pesticides contamination on the emission of PCDD/PCDF to the land from open burning of corn straws. Environ. Pollut., 159, 1744–1748.
- [46] Leroy, B.L.M., Herath, H.M.S.K., Sleutel, S., De Neve, S. et al., 2008. The quality of exogenous organic matter: short-term effects on soil physical properties and soil organic matter fractions. Soil Use Manage., 24, 139–147.
- [47] Xu, Y.P. Lu, P., Tan, F., 2013. Composition and structure of cropland soil fauna in black soil area of Jilin as affected by exogenous carbon and nitrogen (in Chinese). Acta Pedol. Sin., 50, 800–809.
- [48] Glab, T., Kulig, B. 2008. Effect of mulch and tillage system on soil porosity under wheat (*Triticum aestivum*). Soil Till. Res., 99, 169–178.
- [49] Matson, P.A., Parton, W.J., Power, A.G., Swift, M.J., 1997. Agricultural intensification and ecosystem properties. Science, 277, 504–509.
- [50] Abro, S.A., Tian, X., Wang, X., Wu, F. et al., 2011. Decomposition characteristics of maize (*Zea mays. L.*) straw with different carbon to nitrogen (*C/N*) ratios under various moisture regimes. Afr. J. Biotechnol., 10, 10149–10156.
- [51] De Vries, F.T., Manning, P., Tallowin, J.R.B., Mortimer, S.R. et al., 2012. Abiotic drivers and plant traits explain landscape-scale patterns in soil microbial communities. Ecol. Lett., 15, 1230–1239.
- [52] Mendham, D.S., Sankaran, K.V., O'Connell, A.M., Grove, T.S., 2002. Eucalyptus globulus harvest residue management effects on soil carbon and microbial biomass at 1 and 5 years after plantation establishment. Soil Biol. Biochem., 34, 1903–1912.

- [53] Zhong, W.H., Gu, T., Wang, W., Zhang, B. et al., 2010. The effects of mineral fertilizer and organic manure on soil microbial community and diversity. Plant Soil, 326, 511–522.
- [54] Myers, R.T., Zak, D.R., White, D.C., Peacock, A., 2001. Landscape, level patterns of microbial composition and substrate use in upland forest ecosystems. Soil Sci. Soc. Am. J., 65, 359–367.
- [55] Moore-Kucera, J., Dick, R., 2008. PLFA profiling of microbial community structure and seasonal shifts in soils of a Douglas-fir chronosequence. Microb. Ecol., 55, 500–511.
- [56] Bastian, F., Bouziri, L., Nicolardot, B., Ranjard, L., 2009. Impact of wheat straw decomposition on successional patterns of soil microbial community structure. Soil Biol. Biochem., 41, 262–275.
- [57] Song, D.Y., Bai, Z., He, H.B., Xie, H.T. et al., 2008. The response of soil microbial activity to different nutrition conditions (in Chinese). Ecol. Environ., 17, 1216–1220.
- [58] Marinari, S., Masciandaro, G., Ceccanti, B., Grego, S., 2000. Influence of organic and mineral fertilisers on soil biological and physical properties. Bioresour. Technol., 72, 9–17.
- [59] Qiang, X.C., Yuan, H.L., Gao, W.S., 2004. Effect of cropresidue incorporation on soil CO₂ emission and soil microbial biomass (in Chinese). Chin. J. Appl. Ecol., 15, 469–472.
- [60] Zhang, Q.Z., Wu, W.L., Wang, M.X., Zhou, Z.R. et al., 2005. The effects of crop residue amendment and N rate on soil respiration (in Chinese). Acta Ecol. Sin., 25, 2883–2887.
- [61] Högberg, M.N., Högbeg, P., Myrold. D.D., 2007. Is microbial community composition in boreal forest soils determined by pH, C-to-N ratio, the trees or all three? Oecologia, 150, 590–601.
- [62] Mitchell, R.J., Hester, A.J., Campbell, C.D., Chapman, S.J. et al., 2012. Explaining the variation in the soil microbial community: do vegetation composition and soil chemistry explain the same or different parts of the microbial variation? Plant Soil., 351, 355–362.
- [63] Fierer, N., Jackson, R.B., 2006. The diversity and biogeography of soil bacterial communities. Proc. Natl. Acad. Sci., 103, 626–631.
- [64] Lauber, C.L., Hamady, M., Knight, R., Fierer, N., 2009. Pyrosequencing-based assessment of soil pH as a predictor of soil bacterial community structure at the continental scale. Appl. Environ. Microbiol., 75, 5111–5120.
- [65] Lyncb, H.B., Epps, K.Y., Fukami, T., Vitousek, P.M., 2012. Introduced canopy tree species effect on the soil microbial community in a montane tropical forest. Pac. Sci., 66, 141–150.
- [66] Hackl, E., Pfeffer, M., Donat, C., Bachmann, G. et al., 2005. Composition of the microbial communities in the mineral soil under different types of natural forest. Soil Biol. Biochem., 37, 661–671.
- [67] Crecchio, C., Curci, M., Mininni, R., Ricciuti, P. et al., 2001. Short-term effects municipal solid waste compost amendment on soil carbon and nitrogen content, some enzyme activities and genetic diversity. Biol. Fertil. Soils, 34, 311–318.

Journal of Basic Microbiology

32 Ping Lu et al.

- [68] Cayuela, M.L., Sinicco, T., Mondini, C., 2009. Mineralization dynamics and biochemical properties during initial decomposition of plant and animal residues in soil. Appl. Soil Ecol., 41, 118–127.
- [69] Grayston, S.J., Campbell, C.D., Bardgett, R.D., Mawdsley, J.L. et al., 2004. Assessing shifts in microbial community structure across a range of grasslands of differing management intensity using CLPP, PLFA and community DNA techniques. Appl. Soil Ecol., 25, 63–84.
- [70] Zhang, T.L., Li, Z.P., Wang, X.X., 2006. Soil degradation and its eco-environmental impact under highly-intensified agriculture (in Chinese). Acta Pedol. Sin., 34, 843–850.
- [71] Hodge, A., Campbell, C.D., Fitter, A.H., 2001. An arbuscular mycorrhizal fungus accelerates decomposition and

- acquires nitrogen directly from organic material. Nature, 413, 297–299.
- [72] Wu, Y.Q., Jiang, J.Z., Shen, W.K., He, X.L., 2010. Arbuscular mycorrhiza fungi as an ecology indicator for evaluating desert soil conditions. Front. Agric. Chin., 4, 24–30.
- [73] Oehl, F. van der Heijden, M., Jansa, J., Ineichen, K. et al., 2011. Arbuscular mycorrhizal fungi as bio-indicators in Swiss agricultural soils. Agrarforschung Schweiz, 2, 304– 311.
- [74] Allison, V.J., Miller, R.M., Jastrow, J.D., Matamala, R. et al., 2005. Changes in soil microbial community structure in a tallgrass prairie chronosequence. Soil Sci. Soc. Am. J., 69, 1412–1421.